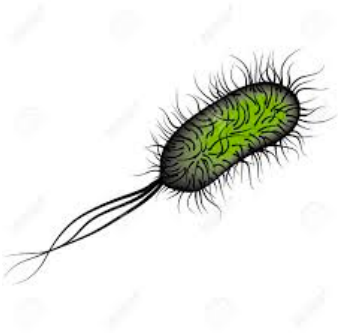

Colony PCR for Screening E. coli

For this procedure, we'll be picking E. coli colonies and using PCR to determine whether they contain our DNA that we've cloned into them. It's good to pick 4-6 colonies to test; for each of these you'll need to set up 2 PCR tubes. We use the PCR tubes that are held in strips of 8 so that you can keep all your reactions together. In one strip, add 25 μl sterile water to each tube. The other strip will be used for your PCR.

1. Pick a single colony using a sterile pipette tip with a pipette set to 5μl and pipette up and down to mix in 25μl of water in a PCR tube. Then transfer 5 μl of this into your empty PCR tube. Do this for about 6 colonies. Save the remaining 20μl for later.

2. The PCR reactions are set up as below. Because you are doing this reaction multiple times, you should make a "Master Mix" of PCR components so you don't have to individually pipette them into tubes every time. This becomes especially time saving when you do this for 10 or more reactions. Make your Master Mix as enough reagent for one more reaction than you are doing, e.g. if you are PCRing 6 colonies, make Master Mix for 7 colonies.

	1X reaction	7X reaction Master
2X GoTaq Green PCR Mix	10 μl	70 μl
10 μM FW primer	1 μl	
10 μM REV primer	1 μl	
water	3 μl	

3. To your tubes containing the 5μl of bacterial cells, add 15μl of your 7x Master Mix. Vortex to mix and spin down in the capsule microfuge.

4. Put your PCR strips in the thermocycler for the following cycle (probably saved as some file called "GoTaq").

	95	5 min
30x:	95	45 s
	55	30 s
	72	1 min per kb
72		10 min

5. Analyze products on a 1% agarose gel. The GoTaq mix already has gel loading dye in it, so you can just directly load 5-10 μl of your PCR reaction into a gel.

6. For all positive bands on the gel, take the rest of the bacterial cells from step 1 and inoculate them into an overnight LB (+antibiotic) for miniprep the next day.